(51) International Patent Classification 7 : A61K 31/445, A61P 3/00	A1	(11) International Publication Number: **WO 00/62779** (43) International Publication Date: 26 October 2000 (26.10.00)

(21) International Application Number: PCT/GB00/01560

(22) International Filing Date: 20 April 2000 (20.04.00)

(30) Priority Data:
9909066.4   20 April 1999 (20.04.99)   GB

(71) Applicant *(for all designated States except US)*: OXFORD GLYCOSCIENCES (UK) LIMITED [GB/GB]; 10 The Quadrant, Abingdon Science Park, Abingdon, Oxfordshire OX14 3YS (GB).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: DWEK, Raymond, A. [GB/GB]; Oxford GlycoSciences (UK) Limited, 10 The Quadrant, Abingdon Science Park, Abingdon, Oxfordshire OX14 3YS (GB). BUTTERS, Terence, D. [GB/GB]; Oxford GlycoSciences (UK) Limited, 10 The Quadrant, Abingdon Science Park, Abingdon, Oxfordshire OX14 3YS (GB). PLATT, Frances, M. [GB/GB]; Oxford GlycoSciences (UK) Limited, 10 The Quadrant, Abingdon Science Park, Abingdon, Oxfordshire OX14 3YS (GB). PRIESTMAN, David [GB/GB]; Oxford GlycoSciences (UK) Limited, 10 The Quadrant, Abingdon Science Park, Abingdon, Oxfordshire OX14 3YS (GB). JEYAKUMAR, Mylvaganam [GB/GB]; Oxford GlycoSciences (UK) Limited, 10 The Quadrant,

Abingdon Science Park, Abingdon, Oxfordshire OX14 3YS (GB).

(74) Agents: LEE, Nicholas, John et al.; Kilburn & Strode, 20 Red Lion Street, London WC1R 4PJ (GB).

(81) Designated States: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published

With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: COMBINATION OF GLUCOSYLCERAMIDE SYNTHESIS INHIBITORS AND GLYCOLIPID DEGRADING ENZYME IN THERAPY
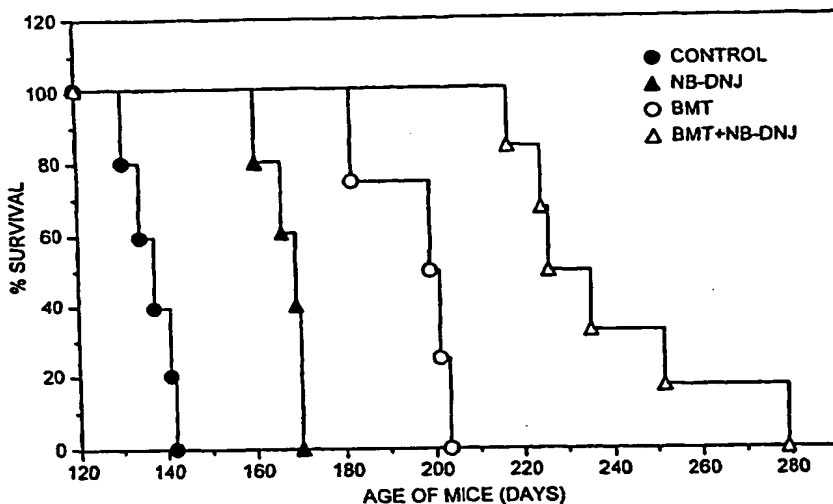
(57) Abstract

The present invention provides the use f an inhibitor of glycolipid synthesis, such as N–butyldeoxynojirimycin (NB–DNJ), N–butyldeoxygalactonojirimycin (NB–DGJ) r N–nonyldeoxynojirimycin (NN–DNJ), and an agent capable of increasing the rate f glycolipid degradation in the manufacture of a medicament for the treatment of a disorder which has at least a component based on glycolipid storage. Such disorders include Gaucher disease, Sandhoff's disease, Fabry's disease. Tay–Sach's disease, Niemann–Pick C storage disease, GM1 gangliosidosis, genetic disorders in which neuronal glycolipid accumulation contributes to the disease's pathology, e.g. mucopolysaccharidoses, neurological disorders in which glucosylceramide–c ntaining glycolipid accumulation contributes to th disease's path logy such as Alzheimer's disease, stroke and epilepsy, cancers of neuronal origin such as glioblastoma and astrocytoma and cancers riginating outside neuronal tissue but presenting with neuronal metastases.

COMBINATION OF GLUCOSYLCERAMIDE SYNTHESIS INHIBITORS AND GLYCOLIPID DEGRADING ENZYME IN THERAPY

The present invention relates to compounds and agents in the manufacture of medicaments for use in the treatment of disorders which have at least a component based

5   on glycolipid storage. Such diseases include Niemann-Pick C storage disease, Gaucher disease, Sandhoff disease, Tay-Sach's disease, GM1 gangliosidosis, Alzheimer's disease, stroke, epilepsy and cancers such as glioblastoma and astrocytoma.

The G_{M2} gangliosidoses are a group of glycosphingolipid (GSL) lysosomal storage

10   diseases which includes Tay-Sachs disease, Sandhoff disease and G_{M2} activator deficiency (Gravel *et al* (1995) in The Metabolic and Molecular Bases of Inherited Disease (Scriver *et al*) Vol 2, pp 2839-79, 3 vols, McGraw Hill, New York). They result from mutations in the genes encoding the hexosaminidase α subunit, β subunit and G_{M2} activator protein respectively. They are characterised by progressive

15   neurodegeneration in response to high levels of lysosomal storage of G_{M2} and related GSLs, in neurones of the central nervous system (CNS) (Gravel *et al* (1995) in The Metabolic and Molecular Bases of Inherited Disease (Scriver *et al*) Vol 2, pp 2839-79, 3 vols, McGraw Hill, New York). There are currently no therapies for these diseases. Potential therapeutic strategies for Tay-Sachs and Sandhoff disease include

20   enzyme augmentation and substrate deprivation (Radin (1996) *Glycoconj. J* 13:153-7; Platt *et al* (1998) *Biochemical Pharmacology* 56:421-30). Augmenting the level of enzyme can be achieved using three clinical strategies, enzyme replacement, bone marrow transplantation or gene therapy.

25   Intravenous administration of mannose-terminated glucocerebrosidase (β-D-glycosyl-*N*-acylsphingosine glucohydrolase, EC 3.2.1.45) is an effective therapy for type 1 Gaucher disease, which is a non-neurological GSL storage disease (Grabowski *et al* (1995) *Ann. Intern. Med.* 122:33-39; Beutler *et al* (1991) *Blood* 78:1183-9). As

glycoprotein enzymes fail to cross the blood-brain barrier, this is not a suitable approach for disease involving GSL storage in the CNS. Bone marrow transplantation has the potential to increase enzyme levels in the periphery, and to a limited extent in the CNS due to secretion of enzyme from cells of bone marrow origin, including microglia (Krivit et al (1995) Cell-Transplant 4:385-392). Results of bone marrow transplantation in GSL lysosomal storage diseases involving storage in the CNS have been mixed (Hoogerbrugge et al (1995) Lancet 345:1398-1402). Partial success was recently reported in a mouse model of Sandhoff disease given syngeneic wild type bone marrow (Norfus et al (1998) J. Clin. Invest. 101:1881-8). This led to increased survival of the mice and improved neurological function. Gene therapy also has promise for treating these diseases, although this is currently experimental (Salvetti et al (1995) Br. Med. Bull 51: 106-122). Substrate deprivation is a potentially generic pharmacological approach for treating the GSL storage diseases (Platt et al (1998) Biochemical Pharmacology 56: 421-30), including the G_{M2} gangliosidoses. This strategy is based upon partial inhibition of the ceramide specific glucosyltransferase (glucosylceramide synthase, UDP-glucose:N-acylsphingosine D-glucosyltransferase, EC 2.4.1.80) which catalyses the first step in GSL biosynthesis (Sandhoff et al (1998) Adv. Lipid Res. 26:119-142). This would reduce the levels of GSLs synthesised so they could be catabolised fully by the residual enzyme activity present in the cells.

Substrate deprivation, utilising the GSL biosynthesis inhibitor N-butyldeoxynojirimycin (NB-DNJ), has previously been tested in an in vitro model of Gaucher disease and shown to prevent storage (Platt et al (1994) J. Biol. Chem. 269:8362-6). NB-DNJ has also been evaluated in an asymptomatic mouse model of Tay-Sachs disease and shown to reduce G_{M2} accumulation in the brain and prevent the neuropathology associated with its storage (Platt et al (1997) Science 276:428-31). NB-DNJ is currently under clinical evaluation in type 1 Gaucher disease.

Defects in ganglioside biosynthesis are found in most human cancers and are thought to underlie the invasive and malignant properties of brain tumours (Hakomori 1996. *Cancer Res*. 56:5309-5318, Fredman et al. 1996 *Glycoconj. J*. 13:391-399).

Glycolipid metabolism also plays a critical role in other neuronal disorders, such as Alzheimer's disease and epilepsy. Niemann-Pick Type C patient neurons present with fibrillar tangles reminiscent of the morphology seen in Alzheimer's disease. Interestingly, GM1 ganglioside binding by amyloid beta-protein induces conformational changes that support its formation of fibrous polymers, and the fibrillar deposition of this protein is an early event in Alzheimer's disease (Yanagisawa et al (1995) *Nat Med* 1:1062-6, Choo-Smith et al (1997) *Biol Chem* 272:22987-90). Thus, decreasing GM1 synthesis could inhibit the fibre formation seen in Alzheimer's disease.

The imino sugar N-butyldeoxynojirimycin (NB-DNJ) is a potent inhibitor of alpha-glucosidase 1 (involved in N-glycan synthesis), and an even more potent inhibitor of glucosylceramide glucosyltransferase. NB-DNJ is currently undergoing clinical trials as a treatment for Gaucher and Fabry diseases, glycolipid storage disorders resulting from mutations in glucocerebrosidase and alpha-galactosidase A, respectively.

We have now found that NB-DNJ administered to mice together with glucocerebrosidase (the major therapy for Gaucher Type I patients) unexpectedly does not compromise the activity of glucocerebrosidase and provides an augmentation of enzyme activity over time due to a protective effect of NB-DNJ on the enzyme. This is surprising as the efficacy of the enzyme would be expected to be compromised in the presence of NB-DNJ as the latter is a weak inhibitor of glucocerebrosidase (IC_{50} = 0.52 mM). Furthermore, we have also found that the co-administration of NB-DNJ with bone marrow transplantation

(to provide enzyme augmentation to increase the rate of neuronal glycolipid degradation) provides an unexpected synergistic effect.

Thus, in a first aspect, the present invention provides the use of an inhibitor of glycolipid synthesis and an agent capable of increasing the rate of glycolipid degradation in the manufacture of a medicament for the treatment of a disorder which has at least a component based on glycolipid storage.

Disorders which result from accumulation/storage of glucosylceramide-containing glycolipids include Gaucher disease, Sandhoff's disease, Fabry's disease, Tay-Sach's disease, Niemann-Pick C storage disease, GM1 gangliosidosis, genetic disorders in which neuronal glycolipid accumulation contributes to the disease's pathology, e.g. mucopolysaccharidoses, neurological disorders in which glucosylceramide-containing glycolipid accumulation contributes to the disease's pathology such as Alzheimer's disease, stroke and epilepsy, cancers of neuronal origin such as glioblastoma and astrocytoma and cancers originating outside neuronal tissue but presenting with neuronal metastases.

In the context of the present invention, the term "inhibitor" is intended to include inhibitors which inhibit glucosylceramide synthesis. It includes molecules such as N-butyldeoxynojirimycin, N-butyldeoxygalactonojirimycin, N-nonyldeoxynojirimycin and other imino sugar-structured inhibitors of glucosylceramide synthesis. However, in addition, it also includes any other inhibitor of glycolipid, especially glucosylceramide, synthesis, including agents such as 1-phenyl-2-decanoylamino-3-morpholino-1-propanol (PDMP), D-threo-1-phenyl-2-decanoylamino-3-morpholino-1-propanol and structurally related analogues thereof. Furthermore, inhibition can also be achieved by the use of genetic approaches, based on the introduction of nucleic acid coding for proteins or peptides capable of inhibiting glycolipid synthesis or antisense sequences or catalytic

RNA capable of interfering with the expression of enzymes responsible for glycolipid and especially glucosylceramide synthesis (e.g. glucosylceramide synthase). A combination of any of the above inhibitors can be used.

5   Agents capable of increasing the rate of glycolipid (preferably but not essentially neuronal glycolipid) degradation include enzymes which degrade glycolipids, e.g. glucocerebrosidase, lysosomal hexoseaminidases, galactosidases, sialidases and glucosylceramide glucosidase, and molecules which increase the activity of such enzymes. In addition, the agent could comprise a nucleic acid sequence (DNA or RNA)
10   which codes for the enzymes mentioned above, i.e. such sequences could be introduced to increase natural production of such enzymes. The agent may even comprise transplanted bone marrow. A combination of the above agents can be used.

       In a second aspect, the present invention provides the use of N-butyldeoxynojirimycin
15   and an agent capable of increasing the rate of glycolipid degradation in the manufacture of a medicament for use in the treatment of a disorder which has at least a component based on glycolipid storage.

       The galactose analogue of NB-DNJ, N-butyldeoxygalactonojirimycin (NB-DGJ), is
20   known to inhibit GSL synthesis *in vitro* as effectively as NB-DNJ, but is more specific in that it does not inhibit α-glucosidase I and II or β-glucocerebrosidase (Platt et al, (1994) *J Biol Chem* 269(43): 27108-14). It is known that only approximately 10% of the serum level of NB-DNJ is present in the cerebrospinal fluid. Accordingly, high systemic doses of NB-DNJ may have to be administered in order to achieve therapeutic levels in the
25   CNS, and may have to be administered for the duration of a patients life. High concentrations of NB-DNJ in humans causes diarrhoea and in mice it causes weight loss and reduces the size of lymphoid organs. Thus, it would be advantageous to have an

inhibitor of glucosylceramide synthesis which does not have these disadvantages of NB-DNJ.

We have now shown that, when administered to healthy mice, the distribution of NB-DGJ *in vivo* is equivalent or superior to that of NB-DNJ and inhibited GSL synthesis. In addition and significantly, NB-DGJ does not appear to cause the side effects associated with NB-DNJ.

Thus, in a third aspect, the present invention provides the use of N-butyldeoxygalactonojirimycin and an agent capable of increasing the rate of glycolipid degradation in the manufacture of a medicament for use in the treatment of a disorder which has at least a component based on glycolipid storage.

In a fourth aspect, the invention provides a product comprising an inhibitor of glycolipid synthesis and an agent capable of increasing the rate of glycolipid degradation as a combined preparation for simultaneous, sequential or separate use in the treatment of a disorder which has at least a component based on glycolipid storage.

For example, it is envisaged that NB-DNJ (or any other inhibitor of glycolipid synthesis) can be administered to a patient with a glycolipid storage disease in order to maintain low levels of glycolipids. If the dosage of NB-DNJ is incorrect for any reason, an agent for increasing the rate of glycolipid degradation can be administered to restore the low levels of glycolipids.

In a fifth aspect, the invention provides a pharmaceutical composition comprising an inhibitor of glycolipid synthesis and an agent capable of increasing the rate of glycolipid degradation.

Methods and processes for the production of N-butyldeoxynojirimycin can be found for example in US-A-4182767, EP-B-0012278, EP-A-0624652, US-A-4266025, US-A-4405714 and US-A-5151519 for example.

5   In other aspects, the present invention provides:

  (a)   a method for the treatment of a disorder which has at least a component based on glycolipid storage which comprises administering to a subject in need thereof a therapeutically effective amount of an inhibitor of glycolipid synthesis and an
10   agent capable of increasing the rate of glycolipid degradation;

  (b)   a method for the treatment of a disorder which has at least a component based on glycolipid storage which comprises administering to a subject in need thereof a therapeutically effective amount of N-butyldeoxynojirimycin and an agent capable of increasing the rate of glycolipid degradation;

15   (c)   a method for the treatment of a disorder which has at least a component based on glycolipid storage which comprises administering to a subject in need thereof a therapeutically effective amount of N- butyldeoxygalactonojirimycin and an agent capable of increasing the rate of glycolipid degradation.

20   The medicaments described herein and which are also for use in the methods provided herein, may include one or more of the following: preserving agents, solubilising agents, stabilising agents, wetting agents, emulsifiers, sweeteners, colorants, odourants, salts, buffers, coating agents or antioxidants. They may also contain therapeutically active agents in addition to the compounds and/or agents described herein.

25

Routes of Administration
The medicaments may be adapted for administration by any appropriate route, for example by the oral (including buccal or sublingual), rectal, nasal, topical (including buccal,

sublingual or transdermal), vaginal or parenteral (including subcutaneous, intramuscular, intravenous or intradermal) route. Such a composition may be prepared by any method known in the art of pharmacy, for example by admixing the active ingredient with a carrier under sterile conditions.

Various routes of administration will now be considered in greater detail:

(i)   Oral Administration

Medicaments adapted for oral administration may be provided as capsules or tablets; as powders or granules; as solutions, syrups or suspensions (in aqueous or non-aqueous liquids); as edible foams or whips; or as emulsions.

Tablets or hard gelatine capsules may comprise lactose, maize starch or derivatives thereof, stearic acid or salts thereof.

Soft gelatine capsules may comprise vegetable oils, waxes, fats, semi-solid, or liquid polyols etc.

Solutions and syrups may comprise water, polyols and sugars. For the preparation of suspensions oils (e.g. vegetable oils) may be used to provide oil-in-water or water-in-oil suspensions.

(ii)   Transdermal Administration

Medicaments adapted for transdermal administration may be provided as discrete patches intended to remain in intimate contact with the epidermis of the recipient for a prolonged period of time. For example, the active ingredient may be delivered from the patch by iontophoresis (Iontophoresis is described in *Pharmaceutical Research*, 3(6):318 (1986)).

(iii) Topical Administration

Medicaments adapted for topical administration may be provided as ointments, creams, suspensions, lotions, powders, solutions, pastes, gels, sprays, aerosols or oils.

For infections of the eye or other external tissues, for example mouth and skin, a topical ointment or cream is preferably used. When formulated in an ointment, the active ingredient may be employed with either a paraffinic or a water-miscible ointment base. Alternatively, the active ingredient may be formulated in a cream with an oil-in-water base or a water-in-oil base.

Medicaments adapted for topical administration to the eye include eye drops. Here the active ingredient can be dissolved or suspended in a suitable carrier, e.g. in an aqueous solvent.

Medicaments adapted for topical administration in the mouth include lozenges, pastilles and mouthwashes.

(iv)   Rectal Administration
Medicaments adapted for rectal administration may be provided as suppositories or enemas.

(v)   Nasal Administration
Medicaments adapted for nasal administration which use solid carriers include a coarse powder (e.g. having a particle size in the range of 20 to 500 microns). This can be administered in the manner in which snuff is taken, i.e. by rapid inhalation through the nose from a container of powder held close to the nose.

Compositions adopted for nasal administration which use liquid carriers include nasal sprays or nasal drops. These may comprise aqueous or oil solutions of the active ingredient.

Medicaments adapted for administration by inhalation include fine particle dusts or mists, which may be generated by means of various types of apparatus, e.g. pressurised aerosols, nebulisers or insufflators. Such apparatus can be constructed so as to provide predetermined dosages of the active ingredient.

5

(vi)   Vaginal Administration

Medicaments adapted for vaginal administration may be provided as pessaries, tampons, creams, gels, pastes, foams or spray formulations.

10   *(vii) Parenteral Administration*

Medicaments adapted for parenteral administration include aqueous and non-aqueous sterile injectable solutions or suspensions. These may contain antioxidants, buffers, bacteriostats and solutes which render the compositions substantially isotonic with the blood of an intended recipient. Other components which may be present in such compositions include water, alcohols, polyols, glycerine and vegetable oils, for example. Compositions adapted for parenteral administration may be presented in unit-dose or multi-dose containers, for example sealed ampoules and vials, and may be stored in a freeze-dried (lyophilised) condition requiring only the addition of a sterile liquid carrier, e.g. sterile water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets.

Dosages

Dosages will be readily determinable by routine trials, and will be under the control of the physician or clinician. The guiding principle for determining a suitable dose will be delivery of a suitably efficacious but non-toxic, or acceptably toxic, amount of material. For NB-DNJ or a similar compound, a daily dosage for an adult could be expected to be in the range of from 1 mg to 2 g of active agent, and may be in the range of from 100 to 800

mg or 300 to 600 mg. The dosage may be administered in a single daily dose or alternatively in two, three or more doses during the day.

Preferred features of each aspect of the invention are as for each of the other aspects

5 *mutatis mutandis*.

In the accompanying drawings:

Figure 1 is a graph plotting % survival against age of Sandhoff mice in days when

10 treated with different agents.

Figures 2A-D are graphs showing the short term distribution of radiolabelled *N*B-DNJ and *N*B-DGJ in mouse. Mice (n = 5 per group) were dissected 90 min after oral administration of [^{14}C]-*N*B-DNJ (open bars) or [^{3}H]-*N*B-DGJ (filled bars). A = total

15 compound in intestine and urine. B = total compound in organs. C = compound concentration in serum. D = compound in organs expressed as a ratio to compound in serum. * denotes a significant difference between the *N*B-DNJ and the *N*B-DGJ treated mice (p < 0.05).

20 Figures 3A-C show glycosphingolipid depletion in mouse liver after feeding *N*B-DNJ or *N*B-DGJ. Gangliosides were purified from liver and separated by TLC. G_{M2} concentration was measured by densitometry of the scanned TLC chromatograms. A = G_{M2} concentration in livers of mice fed 300 – 4800 mg/kg/day *N*B-DNJ (open bars) or *N*B-DGJ (filled bars) for 10 days, (n = 5 per group). B = TLC separated G_{M2} band of

25 livers from mice treated for 5 weeks with 2400 mg/kg/day. C = densitometry of TLC in B. * denotes significantly lower concentration than the control concentration (p < 0.05).

Figure 4 shows the growth of mice fed *N*B-DNJ or *N*B-DGJ. Mice were given 2400 mg/kg/day of *N*B-DNJ (o), *N*B-DGJ (•), or a control diet (☐ N = 10 per group. * denotes a significant difference compared to control weights (p < 0.01).

5   Figure 5 shows the lymphoid organ size in mouse after *N*B-DNJ or *N*B-DGJ treatment. Wet weight of thymus and spleen was determined at dissection after 5 weeks of treatment with 2400 mg/kg/day of *N*B-DNJ (open bars). *N*B-DGJ (filled bars), or a control diet (dashed bars). N = 4 per group. * denotes a significant difference compared to control weights (p < 0.001).

10

Figure 6 shows the inhibition of lactase activity by *N*B-DNJ, *N*B-DGJ, DNJ, and DGJ. Lactase activity expressed as % of control activity at different concentrations of *N*B-DNJ (o), *N*B-DDJ (•), DNJ (☐, and DGJ (■).

15   The invention will now be described with reference to the following examples, which should not in any way be construed as limiting the scope of the invention.

EXAMPLES

20   *Example 1 – Co-administration of Ceredase™ and NB-DNJ*

A group of mice were treated with NB-DNJ at 4800 mg/kg/day for 5 weeks. After a low intravenous dose (5-10 U/kg) of Ceredase™ (Genzyme Corporation) administered as a single injection via the tail vein, serum enzyme activity was measured by taking

25   sequential serum samples from the tail vein to monitor enzyme activity over time. Ceredase™ is a modified form of β glucocerebrosidase. The results are shown in Table 1 below.

Table 1 – Effect of NB-DNJ on circulatory activity and half life of Ceredase™

Mouse	Peak Activity	$T_{1/2}$ (min)
Control 1	5.8	4.2
2	7.9	3.3
3	8.0	1.5
4	6.8	1.8
5	30.0	1.4
6	2.8	2.0
7	13.6	1.2
8	17.6	1.2
Mean ± sem	11.6 ± 3.1	2.1 ± 0.4
NB-DNJ 1	13.9	1.7
2	32.1	4.9
3	24.1	5.3
4	13.1	3.0
5	21.0	3.5
6	68.3	2.4
7	19.2	2.8
Mean ± sem	27.4 ±7.2	3.4 ± 0.5

Ceredase activity and serum half lives appeared to be increased in mice treated with NB-

5   DNJ, suggesting a protective effect of the compound to enzyme clearance. Therefore,

(a) co-administration of NB-DNJ with Ceredase™ does not compromise activity and (b)

there is a surprising augmentation of enzyme activity over time due to a protective effect

of the compound on the enzyme.

Example 2 – Co-administration of NB-DNJ and Bone marrow transplantation in a mouse model of Sandhoff disease

Sandhoff mice were bone marrow transplanted at two weeks of age and drug therapy initiated at 9.5-11 weeks of age (600 mg/kg/day). Survival curves were plotted for each group of animals with each point on the graph representing a death (see Figure 1). The untreated (no BMT, no drug) survived (longest survivor) until 140 days (filled circles), NB-DNJ only (no BMT) survived until 170 days, BMT only (no NB-DNJ) survived until 200 days, and NB-DNJ plus BMT had extended survival from 200-280 days. The data show synergy approximately 13% above additive.

In Examples 3-7 below, the following materials and methods were used:

Animals

Female C57BL/6 mice were housed under standard non-sterile conditions. The mice were provided with water _ad libitum_ and prior to drug administration were fed pelleted chow (expended Rat and Mouse Chow 1, SDS Ltd., Witham, Essex, UK). All experiments were performed on age-matched animals.

Treatment of Mice with NB-DNJ and NB-DGJ

The mice (6 weeks old) were fed a diet of powdered chow (expended Rat and Mouse Chow 3, ground, SDS Ltd.) or diet containing NB-DNJ or NB-DGJ. The diet and compound (both as dry solids) were mixed thoroughly, stored at room temperature, and used within 7 days of mixing. The mice were maintained on NB-DNJ or NB-DGJ at doses of 300 – 4800 mg/kg/day for 10 days, or 2400 mg/kg/day for 5 weeks.

Radiolabelling of NB-DGJ

A galactose oxidase/Na[^{3}H]$_4$B method was used to radiolabel the C6-carbon of NB-DGJ. A solution of NB-DGJ (1.3 mg), galactose oxidase (80 units), and catalase (37000 units)

in 200 µl 10 mM sodium phosphate buffer was incubated for 24h at room temperature whilst stirring. The reaction was stopped by heating the solution to 95°C for 5 min. After centrifuging (10 mins, 13000 rpm), 1M NaOH was added to the supernatant until pH 10-12 was achieved. Na[^{3}H]$_4$B (4.3 mCi) was added and the solution incubated for 2h at 30°C, after which NaBD$_4$ (1 mg) was added and the solution incubated for 1h at 30°C. The solution was neutralised with 1M acetic acid and then dried down. After removing borate by washing with acidified methanol (0.6% glacial acetic acid in methanol) 5-10 times, the [^{3}H]-NB-DGJ mixture was resuspended in water, added to an AG50-column (equilibrated with water) and eluted with 1-4 M NH$_3$. [^{3}H]-NB-DGJ was further purified on HPLC (Dionex CS10 hpcec chromatography, isocratic elution with 50 mM Na$_2$SO$_4$, 2.5 mM H$_2$SO$_4$, 2.5 mM H$_2$SO$_4$, and 5% ACN), and finally the AG50-column step was repeated.

Short-term Distribution of [^{14}C]-NB-DNJ and [^{3}H]-NB-DGJ in Mice

Mice were orally gavaged with 100 µl water containing 25µg (10^6 cpm) [^{14}C]-NB-DNJ or [^{3}H]-NB-DGJ and 1 mg non-radiolabelled NB-DNJ or NB-DGJ, respectively. Urine and faeces were collected over 90 min. After 90 min the mice were killed and the serum, organs, and any additional urine and faeces were collected. Organs were homogenized in a four fold volume of water and faeces in a ten fold volume. Aliquots of 500 µl homogenate, 100 µl urine, or 50 µl serum were mixed with 4 ml scintillation fluid and [^{14}C] or [^{3}H] counts measured. The quenching by the different tissues of both isotopes was determined by measuring the counts of known amounts of radiolabelled compound added to tissue homogenates, and the results were corrected accordingly.

Glycosphingolipid Analysis of Mouse Liver

Liver samples were homogenised in water and lyophilised. Dried homogenates were extracted twice in chloroform: methanol (2:1, v/v), first overnight at 4°C and then for 3h at room temperature, pooled and dried under nitrogen. The extracts were resuspended in

500 µl chloroform: methanol (1:1, v/v), base-treated by adding 83 µl of 0.35 M NaOH in methanol and digested for 90 min at room temperature and partitioned by adding 83 µl water: methanol (9:1, v/v), 166.5 µl water and 416 µl chloroform. The upper phase containing the gangliosides was separated from the lower phase after mixing and low

5   speed centrifugation, and the lower phase was washed twice with Folsh (chloroform: methanol: 0.47% KCl, 3:48:47, v/v). Upper phases were combined, dried down to half volume under nitrogen, dialysed against water, lyophilised and resuspended in chloroform: methanol (2:1, v/v). An equivalent of 5 mg dry weight of tissue was separated by TLC chloroform: methanol: 0.22% $CaCl_2$, 60:35:8, v/v). The TLC plate

10   was air-dried, sprayed with orcinol: sulphuric acid (0.2% (w/v): 2N), and heat-treated (90°C for 10 min). The intensity of bands was quantified by scanning densitometry.

Determination of NB-DNJ and NB-DGJ Concentrations in Serum and Liver

Serum and supernatant of liver homogenate (130 mg/ml in 10% methanol) were

15   centrifuged three times through a Millipore Ultrafree filter, after an internal standard (*N*B-pentylDNJ) had been added to the samples. The pooled filtrates were purified on an HCl preconditioned SCX column, eluted with 1% NH_3 in MeOH, dried down, resuspended in water, further purified on a C18 column (MeOH preconditioning, H_2O wash, and MeOH elution), and finally quantified by HPLC (Dionex CS10 hpcec

20   chromatography, isocratic elution with 50 mM Na_3SO_4, 2.5 mM H_3SO_4, and 5% ACN).

Purification of Disaccharidases and Measurement of Sucrase, Maltase and Lactase Activity

The enzymes sucrase-isomaltase (EC 3.2.1.10/48) and lactase-phlorizin hydrolase (EC

25   3.2.1.62/108) were purified from porcine intestine at 4°C as follows. The intestine (100g) was cut into small pieces, washed by stirring in 250 ml of 150 mM NaCl/10 mM KCl for 30 min, and extracted twice with 125 ml of 2M urea, 50 mM EDTA, and 50 mM KCl at pH 7. The urea extracts were combined and homogenised (Waring blender),

the homogenate was centrifuged at 60,000g for 75 min, and the pellet was resuspended in
50 ml of a solution containing 10 mM EDTA and 10mM L-cysteine-HCl in 50 mM
potassium phosphate buffer at pH 7.5 (pre-equilibrated to 37°C). After addition of
papain (15 units/ml), the mixture was incubated for 30 min at 37°C, and centrifuged at
5   105000g for 60 min. The supernatant was removed and precipitated in 75 ml of ethanol
at -20°C for 1h. The precipitate was recovered by centrifugation at 5000g for 10 min,
dissolved in 5-10 ml of 10 mM potassium phosphate buffer at pH 7.5, and the solution
was centrifuged at 30000g for 60 min. The supernatant was removed and stored at 4°C
in the presence of 0.02% sodium azide. Sucrase, maltase and lactase activity were
10   determined in the enzyme preparation (diluted to a suitable concentration) by incubating
50 µl enzyme. 125 µl sodium citrate buffer (60 mM, pH 6), and 125 µl disaccharide
substrate at 37°C for 30 min, heating to 100°C for 3 min to inactivate the enzyme
centrifuging the mixture at 13000g for 10 min, and determining the glucose concentration
by adding 50 µl of the supernatant to 1 ml trinder reagent (Sigma) and reading the
15   absorbance at 505 nm after 18 min.

Statistical Analysis
Conventional statistical methods were employed to calculate mean values and standard
errors of the mean (S.E.M.). Differences between groups of mice were tested for
20   significance using Student's t-test for unpaired observations. Results in the text and
tables are presented as means ± S.E.M.

Example 3 – Short-term Distribution of [³H]-NB-DGJ and [¹⁴C]-NB-DNJ in Mice

25   The short-term distribution of NB-DGJ and NB-DNJ in mice was determined by giving
the compounds to mice by oral gavage. The radioactive counts in organs, serum, faeces
and urine were measured after 90 min. The concentration of NB-DNJ was 28% higher
than that of NB-DGJ in the total urine collected while in the intestine there was 77%

more *N*B-DGJ than *N*B-DNJ (Fig. 2A). This suggests that *N*B-DGJ passed more slowly out of the gastrointestinal (GI) tract relative to *N*B-DNJ. There appeared to be no difference in distribution of the two compounds in other tissue (Fig.2B). The serum concentration however differed significantly with a lower level of *N*B-DGJ relative to

5   *N*B-DNJ (Fig. 2C), possibly reflecting the slower uptake of *N*B-DGJ from the GI tract. When adjusted for differential serum levels *N*B-DGJ was distributed to the tissue more efficiently than *N*B-DNJ (Fig. 2D).

Example 4 – Long Term Distribution of NB-DGJ and NB-DNJ in Mouse Serum and Liver

10

To assay the steady state levels of the compounds when administered long term via the oral route, the concentrations of *N*B-DGJ and *N*B-DNJ in serum and liver were determined by HPLC after treating mice with 2400 mg/kg/day of *N*B-DNJ or *N*B-DGJ (non-radiolabelled) for 5 weeks (see Table 2 below). Both serum and liver

15   concentration of drug were higher in *N*B-DGJ treated mice compared to *N*B-DNJ treated (66 ± 3.1 µM compared to 51 ± 13.3 µM for serum, and 207 ± 30.6 µM compared to 103 ± 21.2 for liver). The level of *N*B-DGJ in liver compared to that of *N*B-DNJ suggests that *N*B-DGJ is selectively taken up into the liver as compared to *N*B-DNJ. Thus, *N*B-DGJ may enter tissues more efficiently and persist longer than *N*B-DNJ.

20

Table 2 - Concentration of *N*B-DGJ and *N*B-DNJ in serum and liver: Mice were treated with 2400 mg/kg/day of *N*B-DGJ or *N*B-DNJ for 5 weeks (n=2), and the compound concentration in serum and liver was then determined by duplicate runs on HPLC.

	Compound concentration (µM)	
	Serum	Liver
*N*B-DGJ	60 ± 3.1	207 ± 30.6
*N*B-DNJ	51 ± 13.3	103 ± 21.2

Example 5 – Depletion of GSL by NB-DGJ and NB-DNJ

5 The degree of GSL depletion in liver after 10 days or 5 weeks of treatment was compared between mice administered NB-DGJ or NB-DNJ. The livers were chloroform: methanol-extracted, gangliosides were analysed by thin layer chromatography and the G_{M2} band intensity was quantitated by densitometry. The relative G_{M2} concentrations (compared to control mice) in livers of mice treated with a range of NB-DGJ or NB-DNJ

10 doses (300–4800 mg/kg/day) for 10 days show a dose-dependent response to both compounds (see Fig. 3A). There was no significant difference between the G_{M2} depletion achieved by the two compounds at any of the concentrations tested. After longer treatment (2400 mg/kg/day for 5 weeks), the G_{M2} concentrations in livers of mice treated with NB-DNJ or NB-DGJ were reduced to $35 \pm 4\%$ and $26 \pm 11\%$, respectively, in

15 relation to the concentration in control livers (see Figs. 3B and C).

Thus, both analogues (NB-DNJ and NB-DGJ) were shown to be potent inhibitors of GSL biosynthesis *in vivo*. After 10 days of treatment, dose-dependent GSL depletion was seen in livers of mice fed either NB-DNJ or NB-DGJ. The lowest dose causing GSL depletion

20 was 600 mg/kg/day (25% reduction). The highest dose evaluated (4800 mg/kg/day) caused 60–70% depletion. Similar data were obtained with both compounds. Although there is a two fold higher concentration of NB-DGJ in liver this was not observed when GSL depletion was measured, where both compounds gave comparable inhibition of G_{M2} biosynthesis. This may reflect differential cellular uptake of the compounds into

25 hepatocytes, endothelial cells and Kuppfer cells as G_{M2} may be primarily the product of one cell type whereas the compound could be sequestered in non-G_{M2} synthesising cells. GSL depletion after longer treatment at a dosage of 2400 mg/kg/day was also determined. After 5 weeks of feeding, the G_{M2} concentration was reduced by 74% by

NB-DGJ and 65% by NB-DNJ. The drug distribution and G_{M2} depletion suggest treatment of GSL storage disorders should be as effective with NB-DGJ, since it has been shown that NB-DNJ reduces storage in mouse models of these diseases and NB-DGJ is slightly superior to NB-DNJ in inhibiting GSL biosynthesis *in vivo*.

5

Example 6 – Effects of NB-DGJ and NB-DNJ on Growth and Lymphoid Organ Size

To examine the overall well being of the mice treated with NB-DGJ or NB-DNJ
10 (2400 mg/kg/day for 5 weeks) the mice were monitored 2-3 times per week, body weights recorded, and the effects of NB-DGJ and NB-DNJ on growth rates determined (see Fig. 4). The NB-DNJ treated mice grew more slowly than untreated control mice, while NB-DGJ treated mice showed no difference in growth rates relative to the untreated controls. After 5 weeks of treatment, the NB-DNJ mice
15 weighed 25% less than control and the NB-DGJ mice. Thymuses and spleens removed from NB-DNJ mice were smaller than those of control or NB-DGJ mice (see Fig. 5), while the weights of other organs such as liver and kidney were unaffected. Treatment with NB-DNJ reduced the thymus weight by 61 ± 2% and spleen weight by 62 ± 3% compared to organs from control mice. In contrast, NB-
20 DGJ had no effect on lymphoid organ weight. The loss of body weight in NB-DNJ mice did not account for the large reduction in lymphoid organ size. If expressed as a ratio to body weight, the organ weights were still reduced significantly (thymus to body weight ratio was reduced by 45 ± 5% and spleen to body weight ratio by 48 ± 4% in NB-DNJ mice compared to controls). It was observed that NB-DNJ treated
25 mice had less fat associated with their organs (kidney, spleen etc.) and lacked subcutaneous fat compared to control or NB-DGJ treated mice (data not shown).

The fact that loss of body weight and reduction of lymphoid organ size is caused by NB-DNJ but not by NB-DGJ suggests that these effects are a function of glucosidase inhibition (or an as yet unidentified activity) by NB-DNJ, not GSL biosynthesis inhibition (an activity shared by both compounds). The effect of NB-DNJ in the present study on the inhibition of glycogen breakdown could provide a possible explanation for at least part of the weight loss observed in NB-DNJ treated mice. It was shown that, after 12h of starvation, when the control and NB-DGJ treated mice had depleted most of their glycogen, NB-DNJ treated mice still had a significant amount of glycogen in their livers. Both following starvation and between episodes of feeding, the mouse would normally break down glycogen to provide the brain, muscles and other tissues of the body with glucose. However, if glycogenoloysis was partial inhibited, as in the NB-DNJ treated mice, the mouse would have to use other fuel sources, such as fat, to meet its energy demand. The store of adipose tissue would decrease with time resulting in reduced body weight. This hypothesis fits with the observation that the NB-DNJ treated mice (both fed and starved) had very little subcutaneous fat compared to normal or NB-DGJ treated mice. The inhibition of glycogenolysis by NB-DGJ is probably due to inhibition of the glycogen debranching enzyme (4-α-glucanotransferase, EC 2.4.1.25 and α-1,6-glucosidase, EC 3.2.1.33). Although never reported for NB-DNJ, inhibition of the α-1,6-glucosidase activity of this enzyme has previously been observed for other DNJ-derivatives (Arai et al 1998, *Circulation* 97(13): 1290-7; Bollen et al *Eur-J-Biochem* 181(3): 775-80). If this is also the case for NB-DNJ, over prolonged treatment periods this could cause (pathological) glycogen storage. If this does occur however, it is exceeding slow storage as animals on drug for prolonged periods in excess of six months show no overt signs of pathology (data not shown). What may be occurring is that the basal level of glycogen is increased due to partial enzyme inhibition, but that this remains relatively constant over time at the doses of inhibitor used in this study.

NB-DNJ treated mice had consistently smaller lymphoid organs. However, NB-DGJ did not show this effect, again implying that this is not the result of GSL biosynthesis inhibition in animals treated with NB-DNJ.

Example 7 – Inhibition of Disaccharidases In Vitro

NB-DGJ, NB-DNJ and the parental non-alkylated compound DNJ were assessed for their capacities to inhibit the sucrase and maltase activities of the enzyme sucrase-isomaltase (which has disaccharidase activities for the breakdown of sucrose, maltose and isomaltose). Inhibition of this enzyme by DNJ has previously been reported (Hanozet *et al.*, (1981), *J. Biol. Chem* 256:3703-3711). Both substrate and inhibitor concentrations were varied and the K_i calculated (see Table 3). NB-DNJ and DNJ were found to be potent inhibitors of both sucrase and maltase (K_i (sucrase) = 0.03 μM and K_i (maltase)=0.07 μM for DNJ, and K_i (sucrase) = 0.26 μM and K_i (maltase) = 0.37 μM for NB-DNJ), while NB-DGJ was less potent (K_i (sucrase) = 2 mM, (maltase) non-inhibitor at 2 mM).

NB-DNJ, DNJ, NB-DGJ and DGJ were also tested for their capacity to inhibit lactase (Fig. 6 and Table 4). DNJ, NB-DGJ and DGJ all inhibited lactase (K_i of 13 μM, 30 μM and 85 μM for DNJ, DGJ and NB-DGJ, respectively). Lactase inhibition by NB-DNJ was very weak (K_i = 4 mM).

Table 3 – K_is for the inhibition of sucrase and maltase by DNJ, NB-DNJ and NB-DGJ. NI (non-inhibitory at 2mM).

	K_i (μM)	
	Sucrase	Maltase
DNJ	0.03	0.07

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NB-DNJ	0.26	0.37
NB-DGJ	2000	NI

Table 4 - K_is for the inhibition of lactase by DNJ, *N*B-DNJ, DGJ and *N*B-DGJ.

	$K_I (\mu M)$
DNJ	13
NB-DNJ	4000
DGJ	30
NB-DGJ	85

5   The primary side effect of NB-DNJ has been observed to be osmotic diarrhoea. The
diarrhoea is thought to be caused by inhibition of disaccharidases in the intestine, which
means that sugars like sucrose and maltose cannot be catabolised and absorbed from the
digestive system. Sucrose consists of one glucose and one fructose residue, and maltose
of two glucose residues. It is therefore not surprising that the results in this example

10   show that the glucose analogues NB-DNJ and DNJ are very potent inhibitors of the
sucrase and maltase activity while the galactose analogue NB-DGJ is not inhibitory. It
was found that DNJ, NB-DGJ and DGJ all inhibited lactase, but the K_is were at least 10^2
times higher than for sucrase and maltase inhibition by the glucose analogues. NB-DNJ,
however, was not a good inhibitor of lactase (K_i 4mM). In practical terms this means

15   that NB-DGJ might be best tolerated on a lactose-free diet, but should not interfere with
the digestion of other carbohydrates. The lack of side effects associated with NB-DGJ in
vivo may have important implications for the potential treatment of infants and young
children where these side effects could reduce tolerability to a greater extent than those
experienced in adults.

20

Thus it can be seen that *N*B-DGJ has been shown to deplete GSL *in vivo* and to exhibit far fewer *in vitro* and *in vivo* enzyme inhibitory properties than *N*B-DNJ, making this a more selective compound. Of the activities listed below in Table 5, lactase inhibition is the only one associated with *N*B-DGJ and is probably the

5   simplest to overcome by restricting dietary intake of lactose.

Table 5

	NB-DNJ	NB-DGJ
GSL Biosynthesis	+	+
Weight loss	+	-
Lymphoid organ reduction	+	-
ER α-glucosidase I and II inhibition*	+	-
Sucrase and maltase inhibition**	+	-
Lactase inhibition***	-	+

5   *   Platt et al (1994) *J Biol Chem* **269**(43): 27108-14
       ** K_1 (sucrase) = 0.26μM, K_1 (maltase) = 0.37 μM for NB-DNJ
       *** K_1 (lactase) = 85 μM for NB-DGJ
samples (shown in parentheses).

CLAIMS:

1.   The use of an inhibitor of glycolipid synthesis and an agent capable of increasing the rate of glycolipid degradation in the manufacture of a medicament for the treatment of a disorder which has at least a component based on glycolipid storage.

2.   A product comprising an inhibitor of glycolipid synthesis and an agent capable of increasing the rate of glycolipid degradation as a combined preparation for simultaneous, sequential or separate use in the treatment of a disorder which has at least a component based on glycolipid storage.

3.   The use as claimed in claim 1 or product as claimed in claim 2, wherein the inhibitor comprises an imino sugar-structured inhibitor of glycolipid, especially glucosylceramide, synthesis.

4.   The use or product as claimed in any preceding claim, wherein the inhibitor comprises one or more of N-butyldeoxynojirimycin, N-butyldeoxygalactonojirimycin and N-nonyldeoxynojirimycin.

5.   The use or product as claimed in any preceding claim, wherein the inhibitor comprises one or more of 1-phenyl-2-decanoylamino-3-morpholino-1-propanol (PDMP), D-threo-1-phenyl-2-decanoylamino-3-morpholino-1-propanol and structurally related analogues thereof.

6.   The use or product as claimed in any preceding claim, wherein the inhibitor comprises one or more of a nucleic acid coding for a protein or peptide capable of inhibiting glycolipid synthesis, and an antisense sequence or catalytic RNA capable of interfering with the expression of enzymes responsible for glycolipid synthesis.

7.   The use or product as claimed in any preceding claim, wherein the agent capable of increasing the rate of glycolipid degradation comprises one or more of an enzyme which degrades glycolipids, a molecule which increase the activity of such an enzyme, a
5   nucleic acid sequence (DNA or RNA) which codes for such an enzyme, and transplanted bone marrow.

8.   A pharmaceutical composition comprising an inhibitor of glycolipid synthesis and an agent capable of increasing the rate of glycolipid degradation.
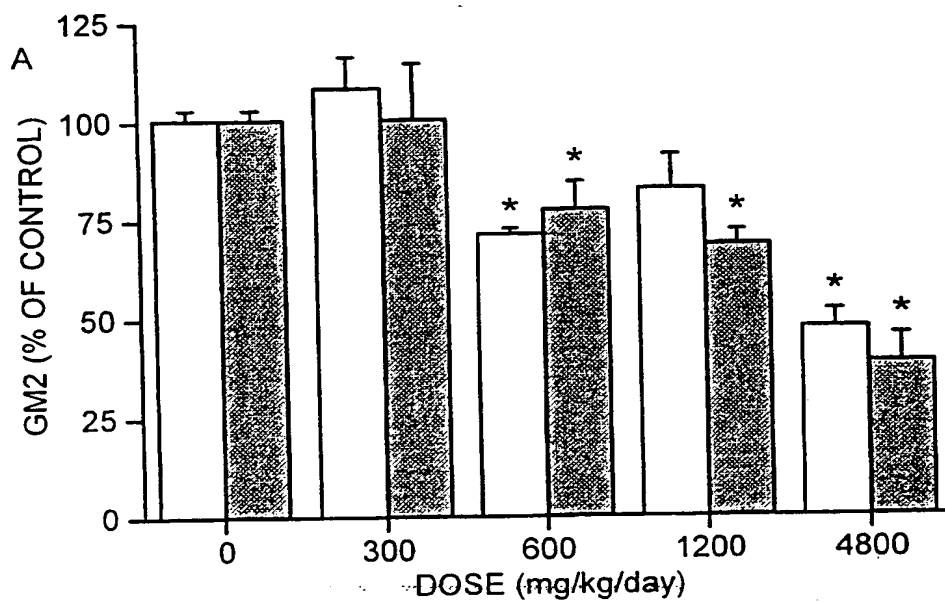
FIG. 1

FIG. 2

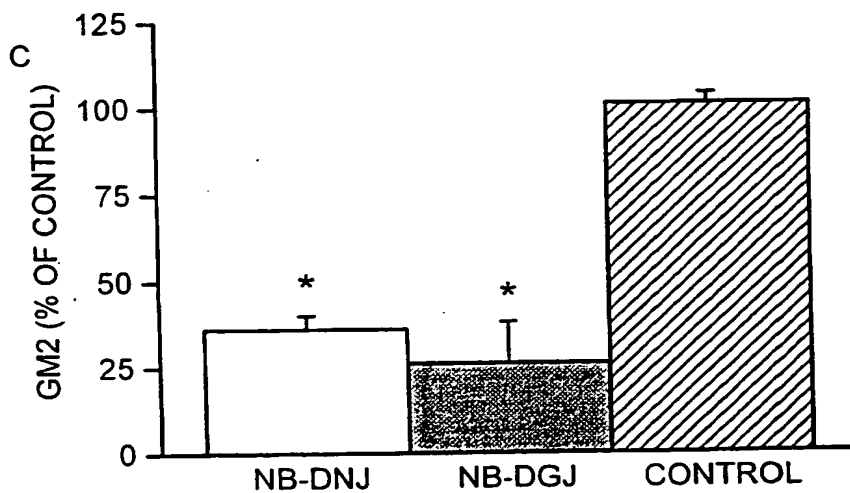FIG. 3

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FIG. 4

5 / 5
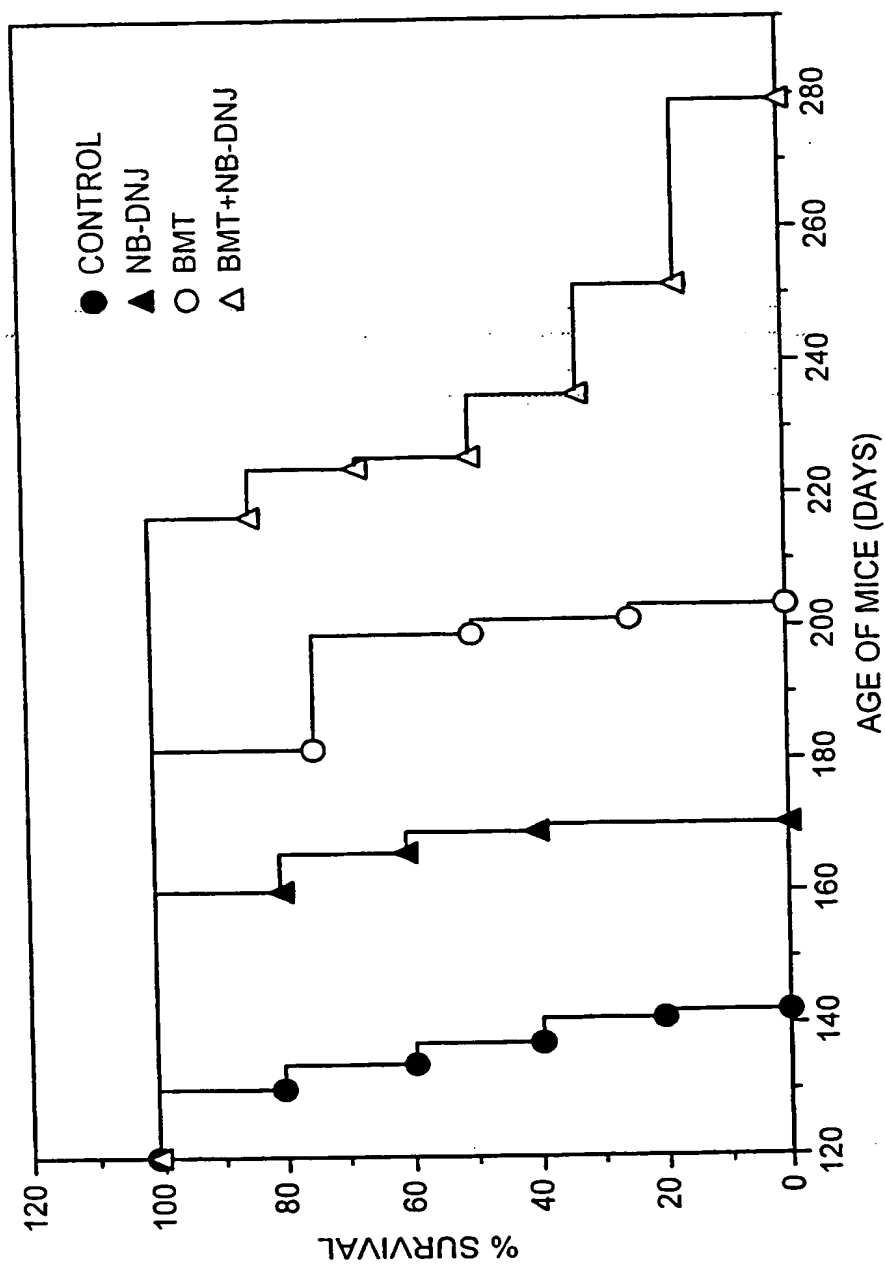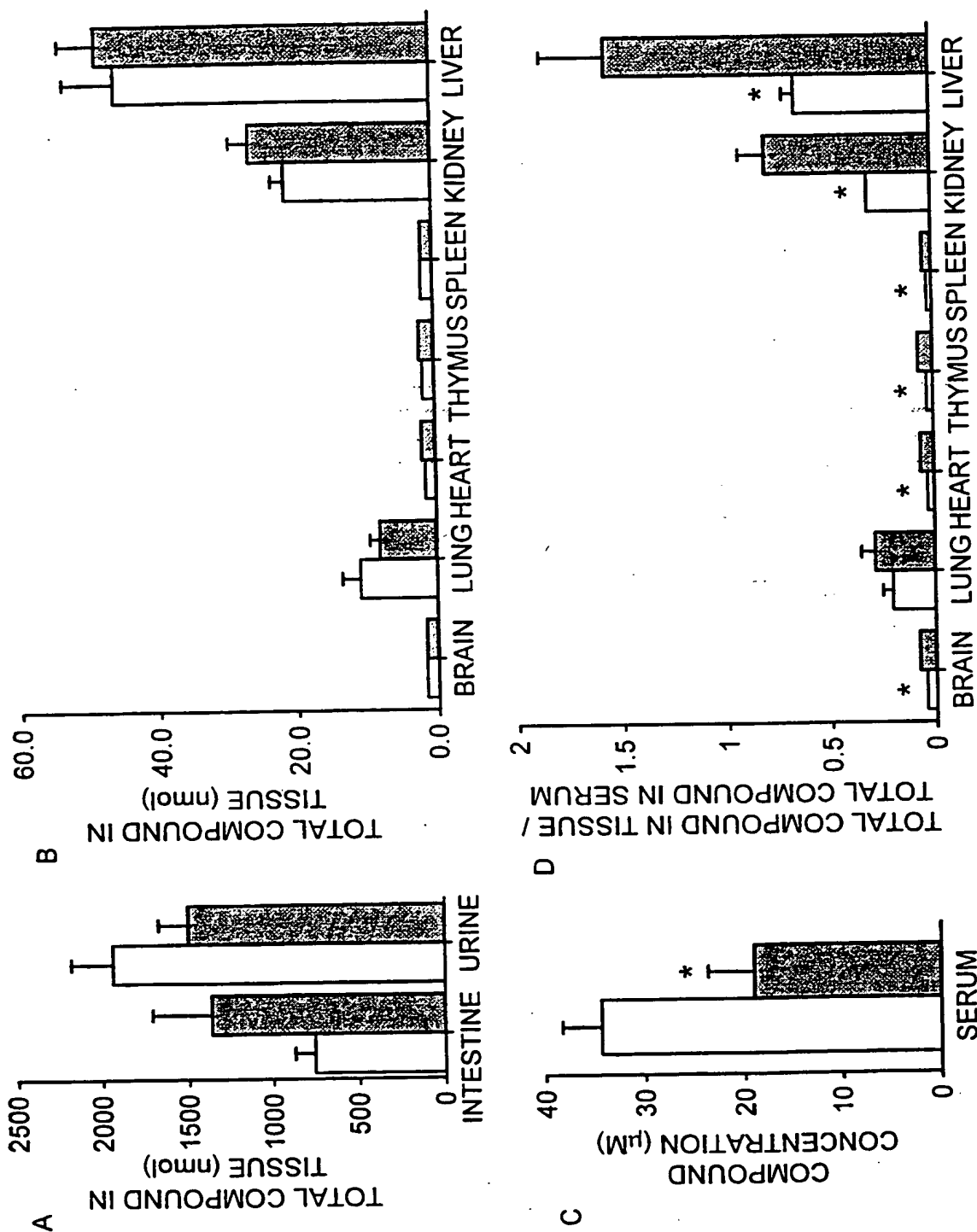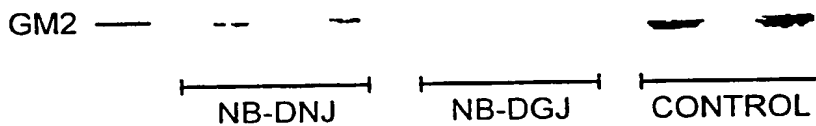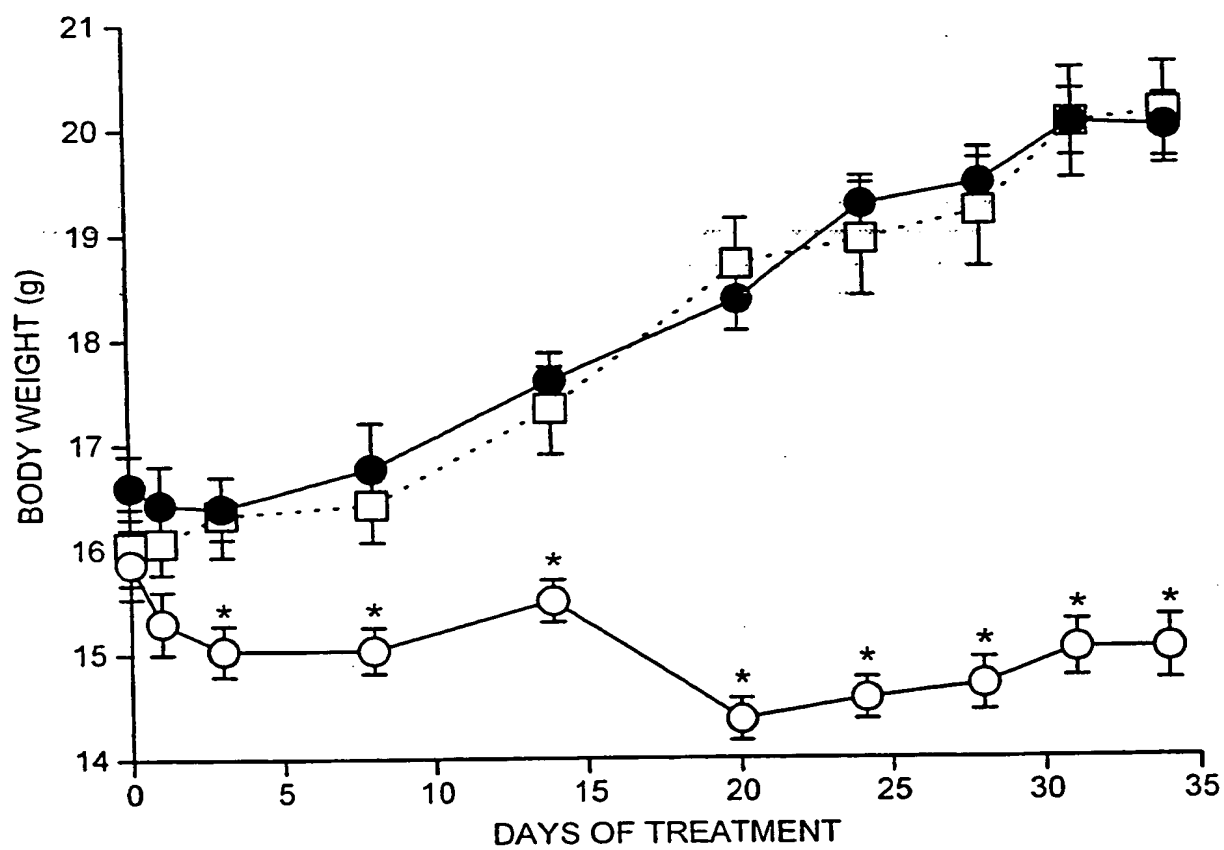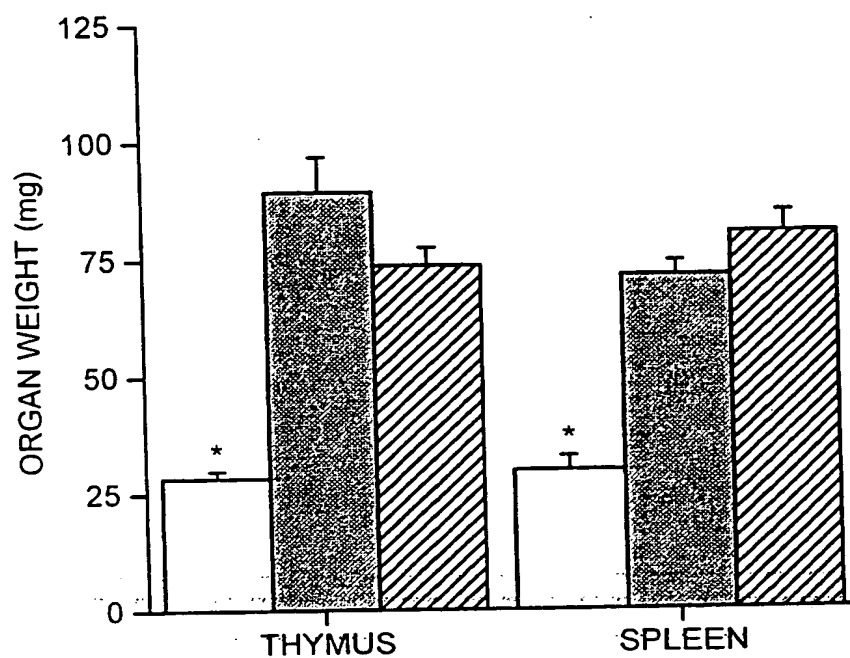
FIG. 5
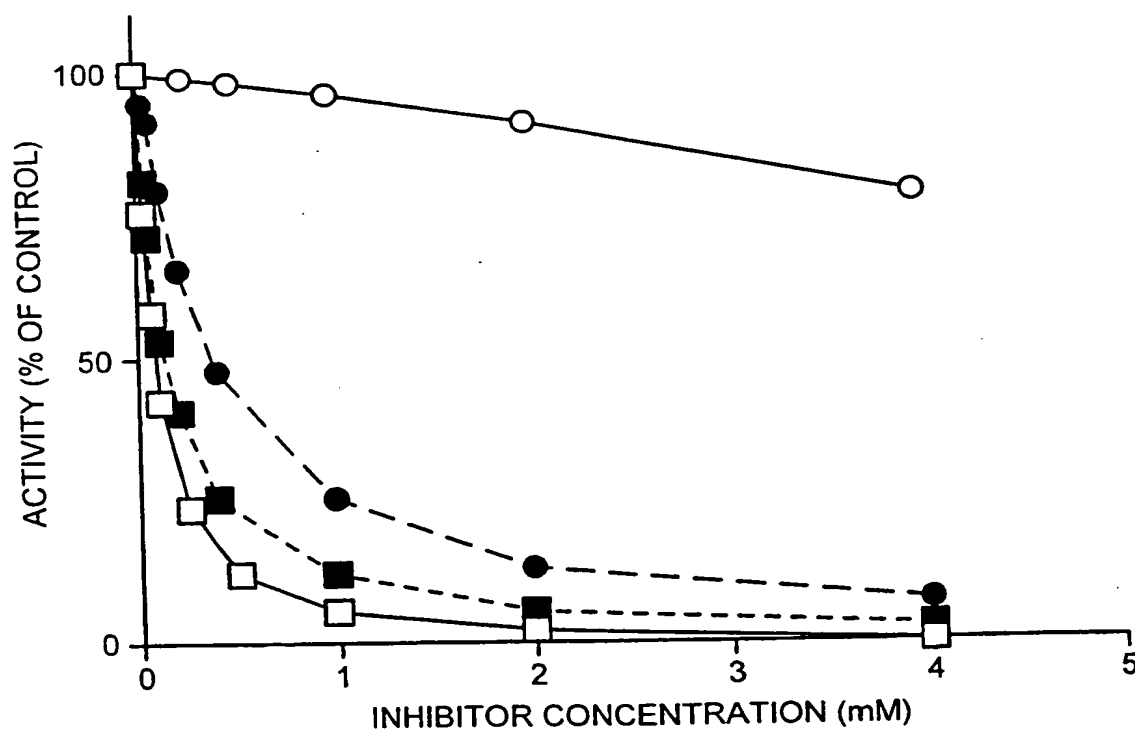
FIG. 6

INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7   A61K31/445   A61P3/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7   A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, MEDLINE, BIOSIS, CHEM ABS Data, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	RADIN N S: "Treatment of Gaucher disease with an enzyme inhibitor." GLYCOCONJUGATE JOURNAL, (1996 APR) 13 (2) 153-7. REF: 22 , XP000929892 abstract page 154, column 1, paragraph 1 page 154, column 2, paragraph 2; figure 1 page 156, column 2 — —/—	1-3,5,8

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

23 August 2000

Date of mailing of the international search report

31/08/2000

Name and mailing address of the ISA
European Patent Office, P.B. 5818 Patentlaan 2
NL – 2280 HV Rijswijk
Tel. (+31–70) 340–2040, Tx. 31 651 epo nl,
Fax: (+31–70) 340–3016

Authorized officer

Gonzalez Ramon, N

INTERNATIONAL SEARCH REPORT

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	PLATT F M ET AL: "NEW THERAPEUTIC PROSPECTS FOR THE GLYCOSPHINGOLIPID LYSOSOMAL STORAGE DISEASES" BIOCHEMICAL PHARMACOLOGY,GB,PERGAMON, OXFORD, vol. 56, no. 4, 1998, pages 421-430, XP000886851 ISSN: 0006-2952 abstract; figure 3 page 425, column 2, paragraph 3	1-5,8
T	ASANO N ET AL: "Sugar-mimic glycosidase inhibitors: natural occurrence, biological activity and prospects for therapeutic application" TETRAHEDRON: ASYMMETRY,NL,ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM, vol. 11, no. 8, May 2000 (2000-05), pages 1645-1680, XP004204568 ISSN: 0957-4166 abstract page 1674 -page 1675	1-6,8
P,X	COX T ET AL: "Novel oral treatment of Gaucher's disease with N-butyldeoxynojirimycin (OGT 918) to decrease substrate biosynthesis." LANCET, (2000 APR 29) 355 (9214) 1481-5. , XP000929894 abstract page 1485, paragraph 3	1-6,8
A	OVERKLEEFT, HERMAN S. ET AL: "Generation of specific deoxynojirimycin-type inhibitors of the non-lysosomal glucosylceramidase" J. BIOL. CHEM. (1998), 273(41), 26522-26527 , XP000934220 abstract; figure 1 page 26527	1-6,8
A	PLATT F M ET AL: "N-BUTYLDEOXYNOJIRIMYCIN IS A NOVEL INHIBITOR OF GLYCOLIPID BIOSYNTHESIS" JOURNAL OF BIOLOGICAL CHEMISTRY,US,AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, BALTIMORE, MD, vol. 269, no. 11, 18 March 1994 (1994-03-18), pages 8362-8365, XP000615445 ISSN: 0021-9258 abstract page 8365	1-6,8

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Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Int tional Application No

PCT/GB 00/01560

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	PLATT F M ET AL: "N-BUTYLDEOXYGALACTONOJIRIMYCIN INHIBITS GLYCOLIPID BIOSYNTHESIS BUT DOES NOT AFFECT N-LINKED OLIGOSACCHARIDE PROCESSING" JOURNAL OF BIOLOGICAL CHEMISTRY,US,AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, BALTIMORE, MD, vol. 269, no. 43, 28 October 1994 (1994-10-28), pages 27108-27114, XP002065777 ISSN: 0021-9258 see discusion abstract	1-6,8
X	WO 98 02161 A (VIANELLO PAOLA ;KOOMEN GERRIT JAN (NL); AERTS JOHANNES MARIA FRANC) 22 January 1998 (1998-01-22) abstract; claims 15,16; figure 4; tables 6,7	1-5,8
E	WO 00 33843 A (UNIV OXFORD ;BUTTERS TERRY D (GB); DWEK RAYMOND A (GB); PLATT FRAN) 15 June 2000 (2000-06-15) abstract; figures 4,7,8	1-6,8

3

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

page 3 of 3

BNSDOCID: <WO 0062779A1 I >

FURTHER INFORMATION CONTINUED FROM   PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.:   1-8

Present claims 1-8 relate to compounds defined by reference to a
desirable characteristic or property, namely "inhibitor of glycolipid
synthesis", "agent capable of increasing the rate of glycolipid
degradation", "nucleic acid coding for a protein capable of inhibiting
glycolipid synthesis", "antisense RNA capable of interfering with
expression of enzymes responsible of glycolipid synthesis", enzyme which
degrades glycolipids", "molecule which increase the activity of such
enzyme", "nucleic acid (DNA or RNA) which codes for such an enzyme"
The claims cover all compounds having this characteristic or property,
whereas the application provides support within the meaning of Article 6
PCT and/or disclosure within the meaning of Article 5 PCT for only a very
limited number of such products/compounds/methods/apparatus. In the
present case, the claims so lack support, and the application so lacks
disclosure, that a meaningful search over the whole of the claimed scope
is impossible. Independent of the above reasoning, the claims also lack
clarity (Article 6 PCT). An attempt is made to define the compounds by
reference to a result to be achieved. Again, this lack of clarity in the
present case is such as to render a meaningful search over the whole of
the claimed scope impossible. The expresion "structurally related
analogues" is vague and indeterminate (claim 5).
Moreover present claims 1-8 relate to a therapeutic application defined
as "a treatment of a disorder which has at least a component based on
glycolipid storage". The definition is not a clear and unequivocal
description of a therapeutic application.
Consequently, the search has been carried out for those parts of the
claims which appear to be clear, supported and disclosed, namely those
parts relating to the compounds as disclosed in the examples, in
particular ceredase and bone marrow transplantation with those
specifically mentioned in claims 4 and 5 in relation to the therapy of
the disorders specified in page 1 (lines 5-7) of the description, with
due regard to the general idea underlying the present application.

The applicant's attention is drawn to the fact that claims, or parts of
claims, relating to inventions in respect of which no international
search report has been established need not be the subject of an
international preliminary examination (Rule 66.1(e) PCT). The applicant
is advised that the EPO policy when acting as an International
Preliminary Examining Authority is normally not to carry out a
preliminary examination on matter which has not been searched. This is
the case irrespective of whether or not the claims are amended following
receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
WO 9802161	A	22-01-1998	AU	3464797 A	09-02-1998
			EP	0912179 A	06-05-1999
WO 0033843	A	15-06-2000	NONE		